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A comparison of genetic variation between an anadromous steelhead, *Oncorhynchus mykiss*, population and seven derived populations sequestered in freshwater for 70 years

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Synopsis

In 1926 cannery workers from the Wakefield Fisheries Plant at Little Port Walter in Southeast Alaska captured small trout, *Oncorhynchus mykiss*, from a portion of Sashin Creek populated with a wild steelhead (anadromous *O. mykiss*) run. They planted them into Sashin Lake which had been fishless to that time and separated from the lower stream by two large waterfalls that prevented upstream migration of any fish. In 1996 we sampled adult steelhead from the lower creek and juvenile *O. mykiss* from an intermediate portion of the creek, Sashin Lake, and five lakes that had been stocked with fish from Sashin Lake in 1938. Tissue samples from these eight populations were compared for variation in: microsatellite DNA at 10 loci; D-loop sequences in mitochondrial DNA; and allozymes at 73 loci known to be variable in steelhead. Genetic variability was consistently less in the Sashin Lake population and all derived populations than in the source anadromous population. The cause of this reduction is unknown but it is likely that very few fish survived to reproduce from the initial transplant in 1926. Stockings of 50–85 fish into five other fishless lakes in 1938 from Sashin Lake did not result in a similar dramatic reduction in variability. We discuss potential explanations for the observed patterns of genetic diversity in relation to the maintenance of endangered anadromous *O. mykiss* populations in freshwater refugia.

Introduction

In recent years many stocks of steelhead, *Oncorhynchus mykiss*, in the western United States have been listed as threatened or endangered under the Endangered Species Act by the National Marine Fisheries Service (Busby et al.[1]). In many cases, freshwater habitat destruction has been cited as a principal factor of population decline and, without substantial habitat restoration, these declines will probably continue. Restoration of freshwater habitats can frequently take years or decades and, in some cases, the continued risk to the remaining population requires some more drastic form of intervention to prevent extinction. In some cases (Flagg et al. 1995, Baugh & Deacon 1988) portions of the wild populations are brought into captivity while habitat restoration efforts are underway. However, the maintenance of wild populations in captivity is fraught with genetic pitfalls. The effective breeding size of these populations is frequently constrained by economics since maintaining captive populations is expensive, and this expense is directly related to the numbers maintained. However, small populations are more subject to genetic change through genetic drift (Falconer 1981), inbreeding depression (Kincaid 1983), domestication selection (Reisenbichler & Brown 1995), and founder effects (Luczynski et al. 1996).

[1] Busby, P.J., T.C. Wainwright, G.J. Bryant, L.J. Lierheimer, R.S. Waples, F.W. Waknitz & I.V. Lagomarsino. 1996. Status review of west coast steelhead from Washington, Idaho, Oregon and California. NOAA Tech. Memo. NMFS-NWFSC-27. 261 pp.

An alternative is to maintain endangered populations in different natural environments that allow for large breeding populations and natural reproduction (Baugh & Decon 1988). This is rarely possible especially for larger animals. For an anadromous species such as *O. mykiss*, normal mortality rates in the marine phase routinely exceed 90%. This high mortality can exceed the reproductive potential of an already endangered stock. To reduce this mortality, pumped seawater systems and marine net-pens are currently used to maintain captive populations (Shaklee et al. 1995), however, this usually involves artificial feeding and captive breeding and, concurrently, the associated genetic risks. If the life cycle of a normally anadromous fish can be completed without the marine phase – and the ability to adapt to seawater is not lost after decades of freshwater sequestration – then large, naturally reproducing populations of endangered, normally anadromous fish might be maintained in protected freshwater habitats until their native habitats are restored. This could reduce some of the genetic concerns (e.g. domestication selection, inbreeding depression) for captive populations.

Long-term genetic change within specific populations has not been studied extensively on a biochemical level because many of the tools we use today (starch gel electrophoresis and DNA sequencing) have only been developed and used extensively in the last two or three decades. Thus, while many populations of animals have been maintained in a captive state for many decades, no genetic record exists of the populations originally brought into captivity. Since most of these captive populations contain relatively small numbers of individuals, gene frequencies would most likely have changed due to founder effects, genetic drift and domestication selection over the decades. If the population has been maintained as a large naturally breeding population in a natural (although perhaps, not native) habitat that has not seen substantial disruption (either anthropogenic or natural), then it is more likely that gene frequencies of 'neutral' alleles might not have changed substantially due to genetic drift and the loss of rare alleles would be minimal. Selection would presumably alter frequencies of alleles with high selection coefficients that were favored in the new environment. While any substantial change in gene frequencies could be seen as undesirable, for some critically endangered populations, the only alternative may be extinction.

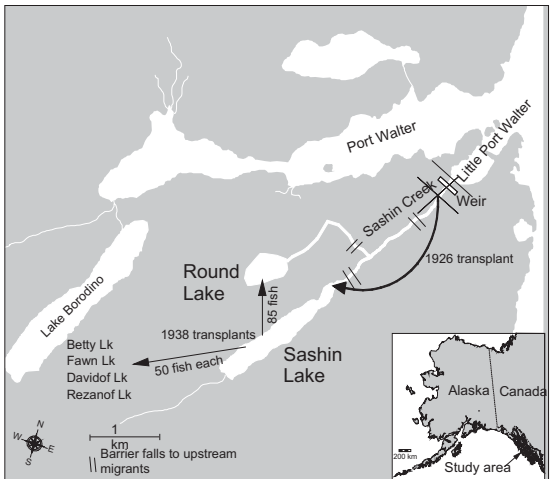

Figure 1. Map of Port Walter showing Sashin Creek study area and indicating the initial transplant (1926) from the anadromous portion of the creek to Sashin Lake and the secondary transplants (1938) to five other barren lakes.

The purpose of this study was to determine if long-term sequestration in fresh water of a normally anadromous stock of fish would result in significant changes in genetic variation that could preclude it as a useful methodology in the preservation of endangered steelhead populations. We compared genetic variation within a wild, anadromous steelhead population (Sashin Creek) in Southeast Alaska with genetic variation in a rainbow trout population from a semi-isolated lake (Sashin Lake) in the same drainage that had been established with a single transplant from the anadromous portion of Sashin Creek in 1926 (70 years earlier) (Anonymous 1939) (Figure 1). We also extended this comparison to include five other lake populations that had been stocked with fish from Sashin Lake in 1938 (approximately 60 years earlier), and a stream population in the intermediate section of Sashin Creek that is separated by barrier falls from the anadromous portion of the creek and Sashin Lake. All of the study lakes and the intermediate stream section were barren of any species of fish at the time of stocking, are above barrier falls that prevent entry of any fish from below, and have no records of subsequent transplants. Fish from all eight populations (hereafter referred to as the 'study' populations) were examined for variation at allozyme and microsatellite loci and mitochondrial DNA haplotypes.

The number of fish originally transplanted to Sashin Lake is unknown. A survey conducted in 1934 indicated the *O. mykiss* population in Sashin Lake to be

large (thousands) so we assumed the initial stocking size was large or survival was quite high in the first generation. Stocking records report numbers stocked for each of the five secondary transplants (Chipperfield[2]) and indicate the maximum breeding size of the secondary transplants was small (50–85 fish). Given that the fish were stocked in July, and thus subject to natural mortality for 10 months prior to first spawning and that the sex ratios at stocking were probably unequal, we hypothesized that founder effects could have substantially altered gene frequencies through loss of rare alleles and increased genetic homozygosity. All of the watersheds in the study area remain pristine and currently support population sizes of at least several hundred to several thousand fish (Thrower, pers. observ.).

Materials and methods

A weir on Sashin Creek was used to capture all adult anadromous steelhead in 1996 and 1997. Hoop nets, minnow traps and sport fishing gear were used to capture resident fish in the study lakes and the intermediate section of Sashin Creek. Tissue samples for DNA extraction consisted of ventral fin clips of live fish. Samples from Sashin Creek and Sashin Lake in 1996 were collected from adult fish and stored in 100% ethanol, whereas those from other populations consisting of mixtures of adults and juveniles were preserved by air drying. Tissue samples for allozyme analysis were collected from a portion of the adult steelhead return in both 1996 and 1997, and from resident fish in 1997 with a separate collection from Sashin Lake made in 1996. The samples from anadromous fish were placed in -20°C freezers for 2 months and transferred to -70°C freezers until processed, whereas resident fish were kept alive during transit to Little Port Walter where tissues were removed and placed on ice for up to 2 h, transferred into liquid nitrogen for up to 3 months, and moved to -70°C freezers until processed.

A seventh lake (Deer Lake), also initially stocked with fish from Sashin Lake, was included for contrast because it is known to have had multiple introductions of fish from outside the study area. At Deer Lake, allozyme samples in the spring of 1997, and DNA samples in the spring of 1998, were collected from fish migrating out of the lake (mostly smolts).

[2] Chipperfield, W.A. 1938. Memo for files, District Ranger U.S. Forest Service, July 30, Juneau, Alaska.

Laboratory analysis

Mitochondrial DNA

A total of 256 fish were examined for mtDNA haplotype variability. DNA was extracted from a small portion of dried fin tissue using Chelex 100 resin (BioRad) following methods given in Nielsen et al. (1994a). We used conserved primers (S-phe and P2) to amplify a highly variable segment of trout mtDNA, including 188 base pairs (bp) of the control region and 5 bp of the adjacent phenylalanine tRNA gene. Double- and single-stranded amplifications were performed using polymerase chain reaction (PCR). PCR products were sequenced directly and the DNA visualized on X-ray film. DNA protocols, sequence for specific primers, and the complete control region segment amplified in *O. mykiss* are given in Nielsen et al. (1994b). Sequences were aligned using MacDNASIS (Hatachi Software Engineering Company, Ltd.).

Microsatellites

Ten nuclear microsatellite loci developed in other laboratories were chosen for this study based on their high level of polymorphism in previous studies of rainbow trout and steelhead in our laboratory. The Omy-series of microsatellite loci were developed specifically for *O. mykiss*; the Oneμ-series was developed for sockeye salmon, *Oncorhynchus nerka*; Ots-series for chinook salmon, *Oncorhynchus tshawytascha*; Sfo-series for brook trout *Salvelinus fontinalis*; and the Ssa-series was developed for Atlantic salmon, *Salmo salar*. For each locus, primer B was labeled according to protocols given in Nielsen et al. (1994b). Amplification of microsatellites followed the methods given in Nielsen et al. (1997) using three fluorescent dyes and running all microsatellite gels on an ABI 373 (Applied Biosystems) adapted for microsatellite analysis. All microsatellite gels were read using ABI Prism Genotyper Software (Applied Biosystems). All loci were initially run individually as separate PCR reactions to determine allelic size distributions in the Alaska rainbow trout. PCR products were then multiplexed on the gels according to the protocol given in Table 1. The size reported here for each microsatellite allele was equal to the size of the total product amplified (including amplified primer sequence). Allelic size was determined by two methods: (1) reference to the ABI Genescan-500 size marker ladder and (2) known *O. mykiss* DNA samples that were rerun on each gel.

Table 1. Multiplex conditions used for amplifications of 10 microsatellite loci in southwest Alaska rainbow trout and steelhead.

	Anneal (°C)	Locus (primer conc.)		
		6Fam-blue	Tet-green	Hex-yellow
Mykiss A	56	One14 (0.14)	Ots1 (0.17) Ssa85 (0.06)	One11 (0.06) Sfo8 (0.10)
Mykiss B	52	Omy77 (0.30) One2 (0.055)	Ssa4 (0.55)	Omy325 (0.11) One8 (0.13)

Primer concentrations are given in parentheses.

Binning of alleles was performed after an analysis of variance for size distributions of each allele at each locus identified by Genotyper. To ensure consistency in both PCR reactions and scoring of microsatellites, 7.8% of all samples were run again on different gels and scored independently. Repeated runs were not included in the analysis of variance performed to establish allelic binning protocols. Alleles found in <5% of the total study population (all samples combined) were considered rare.

Allozymes

Seventy-three allozyme loci known to be variable in *O. mykiss* were screened in 612 fish (Appendix 2). Protein electrophoresis was conducted as described by Aebersold et al.[3] Specific enzyme activities were stained according to Harris & Hopkinson (1976), or Aebersold et al.[3] We followed Reisenbichler & Phelps (1989) and B. Baker (Washington Department of Fish and Wildlife, pers. commun.) for presumed loci for which data were obtained, the tissues in which they were expressed, and the buffer systems with which they were resolved.

Data analysis

To test for a recent genetic bottleneck, an analysis of allozyme and microsatellite data based on Cornuet & Luikart (1996) which examines differences between the observed heterozygosity and expected heterozygosity based on the observed number of alleles using both an infinite alleles model and a stepwise mutation model was conducted on all study populations using BOTTLENECK (version 1.2.02 (16.II.99) Piry et al.[4]).

Results

Analysis of scale samples of anadromous steelhead indicates that smolting takes place at age three or four in Sashin Creek steelhead. The smolts spend 2–3 years at sea and repeat spawners comprise 10–30% of the anadromous adults. Age validation of scale reading on resident fish in Sashin Lake by marking or tagging has not been accomplished, and reliable aging of older fish is difficult; however, resident males mature as early as age two and commonly at age three and females can mature at age three and age four. Maximum age is thought to be at least 8 and possibly substantially older (F. Thrower, unpubl. data).

Mitochondrial DNA

Sashin Lake and all lake populations derived solely from Sashin Lake, and the fish from the intermediate section of Sashin Creek (Sashin Creek residents) were monomorphic for haplotype MYS1. Only the anadromous population collected from Sashin Creek and the Deer Lake population (that had multiple transplants of different origins) showed any variation in the region of the d-loop examined (Table 2). Anadromous steelhead from Sashin Creek had four additional haplotypes and resident fish from Deer Lake had two additional haplotypes. One of the Deer Lake haplotypes (MYS10) was not found in the other study sites.

[3] Aebersold, P.B., G.A. Winans, D.J. Teel, G.B. Milner & F.M. Utter. 1987. Manual for starch gel electrophoresis: A method for the detection of genetic variation. U.S Dept. Commerce NOAA Tech. Rept. NMFS 61. 19 pp.

[4] Piry, S., G. Luikart & J.M. Cornuet. BOTTLENECK: A program for detecting recent effective population size reductions from allele data frequencies. Version 1.2.02 (16.II.1999). Available online. URL: http://www.ensam.inra.fr/URLB/bottlenect/bottleneck.html.

Table 2. Distribution of mtDNA haplotypes in Sashin Creek anadromous steelhead and seven derived landlocked populations.

Population	mtDNA haplotype						Total
	MYS1	MYS3	MYS10	MYS12	MYS21	CLA1	
Sashin Cr. Anadromous	33	3	0	14	5	1	56
Sashin Lake	26	0	0	0	0	0	26
Sashin Cr. Residents	20	0	0	0	0	0	20
Round Lake	20	0	0	0	0	0	20
Betty Lake	20	0	0	0	0	0	20
Davidof Lake	19	0	0	0	0	0	19
Fawn Lake	20	0	0	0	0	0	20
Rezanof Lake	49	0	0	0	0	0	49
Deer Lake	22	3	1	0	0	0	26
Total	229	6	1	14	5	1	256

One anadromous steelhead (designated SCS57) carried a mtDNA sequence highly divergent from the other *O. mykiss* haplotypes found in this study (Table 3). Alignment of this haplotype with other *Oncorhynchus* sequences for the same segment of the mtDNA d-loop, showed close identity between this fish and a coastal cutthroat trout, *O. clarki clarki*, from British Columbia (J. Nielsen, unpubl. data). Only a single variable site differed between the sequence derived from SCB57 and our coastal cutthroat trout. Five additional sites differentiated both the British Columbia coastal cutthroat and SCS57 from a sequence derived from an interior cutthroat (*O. clarki henshawi*) from Nevada.

Microsatellite DNA

All 10 microsatellite loci were variable in at least one of the study populations. Allelic variants ranged from a low of three per locus (One8) to a high of 15 (Ssa85). A total of 111 allelic variants for the 10 loci were detected in the eight study populations (Appendix 1). Of these, 84 were unique (present in only one population) or rare alleles (whose frequencies were less than or equal to 5% of all samples combined). The Deer Lake samples, which were used for contrast, had 17 additional unique alleles. The anadromous Sashin Creek samples contained 24 rare and 35 unique alleles, whereas the Sashin Lake resident population samples contained only 15 rare alleles and one unique allele (Figure 2). When the unique and rare alleles are pooled and adjusted for sample size, the anadromous fish had on average one unique or rare allele per fish, whereas the resident fish had only one unique or rare allele per four fish.

Differences between the Sashin Lake residents and the secondary transplant populations were far less dramatic. The Sashin Creek residents in the intermediate portion of the creek and the Round Lake population had a similar ratio of unique and rare alleles per fish as the Sashin Lake population. The four other lake populations had ratios varying from eight fish per unique or rare allele in the case of Betty Lake to five fish per allele in Rezanof and Davidof lakes to about four fish per allele in the Fawn Lake population.

Distribution of the 27 common alleles was more uniform and did not show a reduction as a result of the initial transplant to Sashin Lake. They ranged from a low of 23 in Betty Lake to highs of 27 in Sashin Lake, Sashin Creek residents and Round Lake, compared to 26 in the anadromous Sashin Creek fish. All the secondary transplant lake populations initiated with 50 fish had lost common alleles (from 1 to 4 per population) compared to the secondary source population (Sashin Lake).

Allozymes

Seventy-three loci were examined of which 18 were found to be variable in at least one of the study populations and the remainder, 52, were invariant (Appendix 3). Within these 18 loci, 40 allelic variants were found in the eight study populations. Only six of these 18 loci were polymorphic (all study populations combined). Fourteen common alleles were detected among this range of loci and populations. There were 13 unique and two rare alleles among the 18 variable loci. The anadromous steelhead sample had eight unique and two rare alleles which, when combined and adjusted for sample size, implies one

Table 3. Mitochondrial DNA sequence (185 base pairs) for MYS1, MYS3, MYS12, MYS21(Alaska), SCS57(Sashin Creek steelhead) and coastal (CLA1) and inland (CLA2) cutthroat trout.

	Base pair																															
Haplotype	1									10									20													
MYS1	T	A	T	A	C	A	T	T	A	A	T	A	A	A	C	T	T	T	A	—	T	G	C	A	C	T	T	T	A			
MYS3	T	A	T	A	C	A	T	T	A	A	T	A	A	A	C	T	T	T	A	—	T	G	C	A	C	T	T	T	A			
MYS12	T	A	T	A	C	A	T	T	A	A	T	A	A	A	C	T	T	T	A	—	T	G	C	A	C	T	T	T	A			
MYS21	T	A	T	A	C	A	T	T	A	A	T	A	A	A	C	T	T	T	A	—	T	G	C	A	C	T	T	T	A			
SCS57	T	A	T	A	C	A	T	T	A	A	T	A	A	A	C	T	T	T	A	G	T	G	C	A	C	T	T	T	A			
CLA1	T	A	T	A	C	A	T	T	A	A	T	A	A	A	C	T	T	T	A	G	T	G	C	A	C	T	T	T	A			
CLA2	T	A	T	A	C	A	T	T	A	A	T	A	A	A	C	T	T	T	A	G	T	G	C	A	C	T	T	T	A			
	30										40										50											
MYS1	—	—	G	C	A	T	T	T	G	G	C	A	C	C	G	A	C	A	G	C	G	C	T	G	T	A	A	T	G	C		
MYS3	—	—	G	C	A	T	T	T	G	G	C	A	C	C	G	A	C	A	G	C	G	C	T	G	T	A	A	T	G	C		
MYS12	—	—	G	C	A	T	T	T	G	G	C	A	C	C	G	A	C	A	G	C	G	C	T	G	T	A	A	T	G	C		
MYS21	—	—	G	C	A	T	T	T	G	G	C	A	C	C	G	A	C	A	G	C	G	C	T	G	T	A	A	T	G	C		
SCS57	T	A	G	C	A	T	T	T	G	G	C	A	C	C	G	A	C	A	A	C	G	C	T	G	T	G	G	T	G	C		
CLA1	T	A	G	C	A	T	T	T	G	G	C	A	C	C	G	A	C	A	A	C	G	C	T	G	T	G	G	T	G	C		
CLA2	T	A	G	C	A	T	T	T	G	G	C	A	C	C	G	A	C	A	A	C	G	C	T	G	T	G	G	T	A	C		
	60										70										80											
MYS1	G	T	A	C	A	C	T	T	T	C	A	T	A	A	A	T	A	A	A	G	T	A	T	A	C	A	T	T	A	A		
MYS3	G	T	A	C	A	C	T	T	T	C	A	T	A	A	A	T	A	A	A	G	T	A	T	A	C	A	T	T	A	A		
MYS12	G	T	A	C	A	C	T	T	T	C	A	T	A	A	A	T	A	A	A	G	T	A	T	A	C	A	T	T	A	A		
MYS21	G	T	A	C	A	C	T	T	T	C	A	T	A	A	A	T	A	A	A	A	T	A	T	A	C	A	T	T	A	A		
SCS57	G	T	A	C	A	C	T	T	T	C	A	T	A	A	A	T	A	A	A	G	T	A	T	A	C	A	T	T	A	A		
CLA1	G	T	A	C	A	C	T	T	T	C	A	T	A	A	A	T	A	A	A	G	T	A	T	A	C	A	T	T	A	A		
CLA2	G	T	A	C	A	C	T	T	T	C	A	T	A	A	A	T	A	A	A	G	T	A	T	A	C	A	T	T	A	A		

	90										100										110									
MYS1	T	A	A	A	C	T	T	T	C	G	A	T	C	C	A	C	T	T	T	G	T	A	G	C	A	C	C	T	A	G
MYS3	T	A	A	A	C	T	T	T	C	G	A	T	C	C	A	C	T	T	T	G	T	A	G	C	A	C	C	T	A	G
MYS12	T	A	A	A	C	T	T	T	C	G	A	[C]	C	C	A	C	T	T	T	G	T	A	G	C	A	C	C	T	A	G
MYS21	T	A	A	A	C	T	T	T	C	G	A	T	C	C	A	C	T	T	T	G	T	A	G	C	A	C	C	T	A	G
SCS57	T	A	A	A	C	T	T	T	C	G	[G]	[C]	C	C	[C]	C	T	T	[C]	G	T	A	G	C	A	[T]	C	T	[G]	G
CLA1	T	A	A	A	C	T	T	T	C	G	[G]	[C]	C	C	[C]	C	T	T	[C]	G	T	A	G	C	A	[T]	C	T	[G]	G
CLA2	T	A	A	A	C	T	T	T	[T]	G	A	[C]	C	C	[C]	C	T	T	[C]	G	T	A	G	C	A	[T]	C	T	A	G
	120										130										140									
MYS1	C	A	C	C	G	A	C	A	A	C	G	C	T	G	T	T	A	T	C	A	A	T	G	C	C	A	T	T	T	C
MYS3	C	A	C	C	[A]	A	C	A	A	C	G	C	T	G	T	T	A	T	C	A	A	T	G	C	C	A	T	T	T	C
MYS12	C	[C]	C	C	G	A	C	A	A	C	G	C	T	G	T	T	A	T	C	A	A	T	G	C	C	A	T	T	T	C
MYS21	C	A	C	C	G	A	C	A	A	C	G	C	T	G	T	T	A	T	C	A	A	T	G	C	C	A	T	T	T	C
SCS57	C	A	C	C	G	A	C	A	A	C	[A]	C	T	G	[A]	T	A	T	[T]	A	A	T	[A]	C	C	A	T	T	T	C
CLA1	C	A	C	C	G	A	C	A	A	C	[A]	C	T	G	[A]	T	A	T	C	A	A	T	[A]	C	C	A	T	T	T	C
CLA2	C	A	C	C	G	A	C	A	A	C	[A]	C	T	G	[A]	T	A	T	C	A	A	T	[A]	C	C	A	T	T	T	C
	150										160										170									
MYS1	C	A	C	G	C	A	C	A	G	C	C	C	G	C	C	G	C	T	G	A	C	G	T	A	G	C	T	T	A	A
MYS3	C	A	C	G	C	A	C	A	G	C	C	C	G	C	C	G	C	T	G	A	C	G	T	A	G	C	T	T	A	A
MYS12	C	A	C	G	C	A	C	A	G	C	C	C	G	C	C	G	C	T	G	A	C	G	T	A	G	C	T	T	A	A
MYS21	C	A	C	G	C	A	C	A	G	C	C	C	G	C	C	G	C	T	G	A	C	G	T	A	G	C	T	T	A	A
SCS57	C	A	C	G	C	A	C	A	[A]	C	C	C	[A]	C	[T]	G	C	T	[A]	[G]	C	G	T	A	G	C	T	T	A	A
CLA1	C	A	C	G	C	A	C	A	[A]	C	C	C	[A]	C	[T]	G	C	T	[A]	[G]	C	G	T	A	G	C	T	T	A	A
CLA2	C	A	C	G	C	A	C	A	[A]	C	C	[T]	G	C	[T]	G	C	T	[A]	[G]	C	G	T	A	G	C	T	T	A	A
	180																													
MYS1	C	T	A	A	A	G																								
MYS3	C	T	A	A	A	G																								
MYS1	C	T	A	A	A	G																								
MYS2	C	T	A	A	A	G																								
SCS5	C	T	A	A	A	G																								
CLA1	C	T	A	A	A	G																								
CLA2	C	T	A	A	A	G																								

Variable nucleotide locations are outlined in comparison with MYS1.

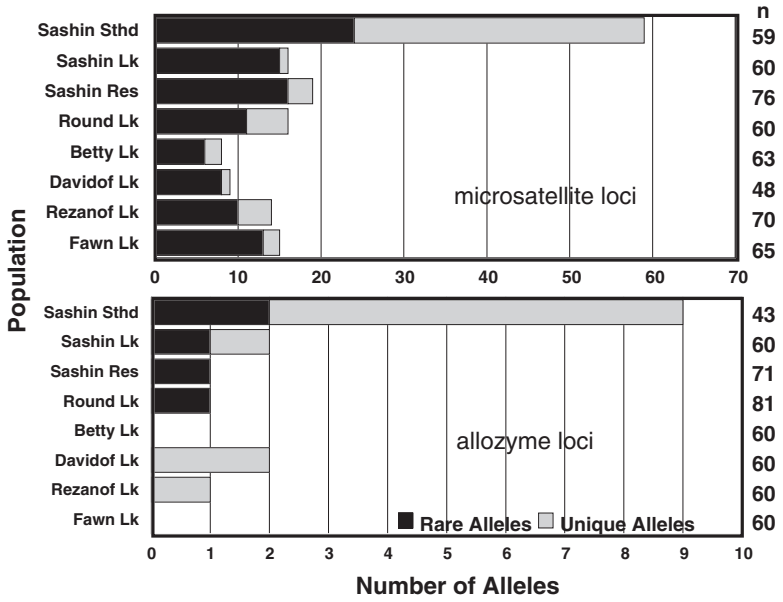

Figure 2. Incidence of unique and rare microsatellite and allozyme alleles in Sashin Creek steelhead and seven derived freshwater populations.

unique or rare allele per four fish. The sample from the primary transplant population, Sashin Lake, had two unique and one rare alleles which, when adjusted for sample size, implies only one unique or rare allele per 40 fish or about one order of magnitude fewer than in the anadromous steelhead (Figure 2). For the secondary transplants, the range of unique and rare alleles varied from zero (Betty and Fawn lakes and Sashin Creek residents) to one each in Rezanof and Round lakes and two in Davidof Lake. Since the unique alleles found in the secondary populations were at low frequencies, and because relatively little time (60 years; 15 generations) has passed from transplantation, it is likely these alleles were present in the source population(s) at low frequency and are not new mutations.

The anadromous steelhead sample contained all of the 14 common alleles. The Sashin Lake sample was lacking only one common allele as were Betty, Davidof and Round lakes. All the other study population samples had the full complement of common alleles. In contrast, the Deer Lake sample had variation in two additional loci (mAAT-2 and ADA-1) both of which were fixed in the study samples. The Deer Lake sample also had three unique alleles in the variable loci and were fixed at two of the polymorphic loci. The 'Bottleneck' analysis did not indicate a heterozygosity excess in any of the populations.

Discussion

The genetic variability of the Sashin Lake population is relatively low when compared to the ancestral, anadromous steelhead population of lower Sashin Creek. The mtDNA evidence indicates perhaps as few as three and probably no more than 7 or 8 females successfully reproduced to start the new population in Sashin Lake, unless the ancestral haplotype proportions were dramatically different from the recent samples. Even with this restriction, the population expanded rapidly in the new habitat and has remained at a relatively large size since at least 1934 when first inventoried. The genetic evidence provided by all three techniques used supports the transplant records of single transplants of Sashin Lake fish into the five other study lakes. The unique mtDNA haplotypes, microsatellite and allozyme alleles found in the Deer Lake population, that was known to have had at least one additional transplant from a source other than Sashin Lake, also supports the lack of additional successful transplants to the study lakes. Virtually all of the lake populations showed some reductions in genetic variability when compared to the donor Sashin Lake population. These reductions range from a loss of unique and rare alleles of approximately 50% (Betty Lake) to virtually no change in frequency (Round Lake).

All five lakes currently have robust populations of naturally reproducing fish. These transplants of 50 and 85 fish appear to have been much more successful at transferring genetic variation than the original transplant into Sashin Lake from lower Sashin Creek. No heterozygosity excess (Cornuet & Luikart 1996) was found in any of the study populations which indicates that recent population bottlenecks have not occurred.

While survival in a quality habitat without competitors or piscine predators cannot be considered the same as that in the original habitat, the establishment of a large, free breeding population is perhaps the most essential element in the preservation of endangered species. This has not been possible with many species of large endangered mammals and some species of fish (Baugh & Deacon 1988, Flagg et al. 1995). However in the case of endangered steelhead, it does appear possible that large populations could be maintained under quasi-natural conditions in freshwater habitats for decades while their native habitat is restored and still have much of the original genetic variation of the population preserved for reintroduction efforts. In fact, in many places in California and the Pacific Northwest, important reservoirs of ancestral steelhead genetic information may still exist behind many irrigation and hydroelectric projects that were put in place in the 1800s and 1900s with no allowance for fish passage. Unfortunately, many of these populations may have been genetically compromised with introductions of stocks of fish from other areas; however, many uncontaminated populations undoubtedly still exist. The fact that these populations have not had the opportunity to express anadromous behavior for decades does not mean that the ability to reinitiate that life history type successfully under the proper conditions has been lost permanently. In fact, the Sashin Lake population and the populations of all the other study lakes, still produce fish that smolt and migrate to sea and return as mature adults to the base of waterfalls blocking access to their natal lakes. Obviously, with complete selection against anadromy in the lake populations, and no reinforcing selection in the original habitat for decades, it is likely survival of the reintroduced fish would be somewhat compromised when compared to the original endemic stock. If a large reservoir of genetic variation has been maintained, successful reinitiation of the anadromous life stage seems likely.

While the use of natural freshwater habitats for the maintenance of a normally anadromous species or stock is not preferable to the use of the original habitat, a naturally reproducing population in a wild or semi-wild state has substantial advantages over maintaining captive populations. However, the effects of freshwater sequestration for decades on the ability of a normally anadromous stock to recolonize its native habitat are unknown and should be investigated. Some evidence for reduced ability to recolonize the former native habitat does appear to exist in the case of the Sashin Lake fish. After 70 years, fish from Sashin Lake and steelhead from Sashin Creek still have substantial genetic differences despite the continued movement of fish from the upper watershed to the lower one. Downstream movement occurs through the normal smolting process of some portion of the upper watershed fish and the downstream movement of fry and juveniles through displacement and washout by floods. Using a Bayesian analysis for stock mixtures of the genotypes present in the watershed, and using the allozyme and microsatellite data independently, Pella & Masuda (2001) concluded that 25% of the anadromous adults at the Sashin Creek weir in 1996 and 1997 had originated in the upper watershed. If this proportion is typical, and the fish of upstream origin mated randomly with those of the anadromous section and offspring survival was similar, then one would expect the genetic profile of the Sashin Creek steelhead to be very similar to the three upstream populations (Sashin Lake, Round Lake and Sashin Creek residents) after 70 years of immigration (Falconer 1981, p. 22). Because differences remain (e.g. frequencies of unique and rare alleles, mitochondrial haplotypes, and PGK-2 alleles), it seems likely that non-random mating and/or differential survival of offspring could be influencing the maintenance of population differences. Research is currently underway at the Little Port Walter Research Station to determine the cause of the maintenance of these stock distinctions.

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Appendix 1. Allele distributions for 10 microsatellite loci isolated in Sashin Creek steelhead and seven derived populations and Deer Lake.

Population	Base	Locus – Oneμ14											
	143	147	149	151	153	155	157	159	161	163	171	173	Total
Sashin Cr.sthd.	0	46	1	13	4	37	1	0	7	3	2	0	114
Sashin Cr. res.	0	93	0	0	0	57	0	0	0	0	0	0	150
Sashin Lake	0	68	0	2	2	43	0	0	1	0	0	0	116
Round Lake	1	74	7	0	0	28	0	0	0	0	0	0	110
Betty Lake	0	84	1	0	0	31	0	0	0	0	0	0	116
Fawn Lake	0	75	0	0	0	55	0	0	0	0	0	0	130
Rezanof Lake	0	89	0	0	0	34	0	3	0	0	0	2	128
Davidof Lake	0	78	0	0	0	18	0	0	0	0	0	0	96
Deer Lake	0	35	0	10	0	5	0	0	0	0	0	0	50
Total	1	642	9	25	6	308	1	3	8	3	2	2	1010

		Locus – Ssa85																	
	97	101	105	109	117	119	121	123	125	127	129	133	135	137	145	157	159	169	Total
Sashin Cr.sthd.	0	0	23	6	0	0	0	2	0	9	5	59	4	2	0	0	0	0	110
Sashin Cr. res.	0	0	42	0	1	0	1	0	0	0	1	101	0	0	4	0	0	0	150
Sashin Lake	0	1	32	2	0	0	0	0	0	1	1	81	0	0	0	0	0	0	118
Round Lake	0	0	54	0	0	0	0	0	0	0	4	60	0	0	0	0	0	0	118
Betty Lake	1	0	56	0	0	0	0	0	0	0	0	65	0	0	0	0	0	0	122
Fawn Lake	0	0	66	0	0	6	0	0	0	0	1	55	0	2	0	0	0	0	130
Rezanof Lake	0	0	16	0	0	1	0	0	0	0	1	114	0	0	0	0	0	0	132
Davidof Lake	0	0	35	0	0	0	0	0	1	0	2	54	0	0	0	0	0	0	92
Deer Lake	0	0	39	0	0	0	0	0	0	1	5	0	1	0	0	2	1	3	52
Total	1	1	363	8	1	7	1	2	1	11	20	589	5	4	4	2	1	3	1024

		Locus – Ots1													
	157	159	161	163	165	167	169	171	173	175	177	237	239	241	Total
Sashin Cr.sthd.	0	0	0	69	2	24	0	0	18	1	1	2	1	0	118
Sashin Cr. res.	0	0	0	58	4	69	2	1	9	0	0	0	0	1	144
Sashin Lake	0	0	0	57	0	48	0	0	10	0	0	0	0	1	116
Round Lake	0	0	0	56	0	22	0	0	12	0	0	0	0	30	120
Betty Lake	0	0	0	27	2	87	0	1	5	0	0	0	0	0	122
Fawn Lake	0	0	0	24	0	95	0	0	10	1	0	0	0	0	130
Rezanof Lake	0	0	0	77	3	40	0	0	0	0	0	0	0	0	120
Davidof Lake	0	2	0	70	0	4	0	0	0	0	0	0	0	18	94
Deer Lake	4	0	7	3	2	36	0	0	0	0	0	0	0	0	52
Total	4	2	7	441	13	425	2	2	64	2	1	2	1	50	1016

Appendix 1. (*Continued*)

Population	Base	Locus – Oneμ11							Locus – Sfo8						Locus – Oneμ8				
	109	119	143	145	147	149	Total		172	174	176	178	Total		157	159	161	173	Total
Sashin Cr.sthd.	1	0	10	43	24	40	118		117	1	0	0	118		0	3	98	1	102
Sashin Cr. res.	0	0	9	52	48	41	150		151	0	1	0	152		0	4	145	1	150
Sashin Lake	0	0	0	41	33	44	118		113	0	1	0	114		0	0	114	0	114
Round Lake	0	2	3	23	17	65	110		114	0	0	0	114		0	4	116	0	120
Betty Lake	0	0	0	6	6	114	126		118	0	0	2	120		0	2	114	0	116
Fawn Lake	0	0	6	54	47	1	108		129	1	0	0	130		0	0	130	0	130
Rezanof Lake	0	0	1	57	18	58	134		130	0	0	0	130		0	2	128	0	130
Davidof Lake	0	0	0	24	2	70	96		96	0	0	0	96		0	0	94	0	94
Deer Lake	0	0	0	28	1	23	52		Not run						4	19	29	0	52
Total	1	2	29	328	196	456	1012		968	2	2	2	974		4	34	968	2	1004

		Locus – Omy77												
	95	99	103	107	115	117	119	121	127	129	131	137	145	Total
Sashin Cr.sthd.	0	0	24	3	17	1	38	9	0	5	15	6	0	118
Sashin Cr. res.	0	0	69	0	0	0	47	0	0	0	28	0	0	144
Sashin Lake	0	0	56	0	0	0	30	0	0	0	34	0	0	120
Round Lake	0	0	20	0	0	0	62	1	0	0	36	0	1	120
Betty Lake	0	0	97	0	0	0	29	0	0	0	0	0	0	126
Fawn Lake	1	0	15	0	0	0	39	0	0	1	58	0	0	114
Rezanof Lake	0	0	104	0	0	0	34	0	0	0	2	0	0	140
Davidof Lake	0	0	45	0	0	0	32	0	0	0	15	0	0	92
Deer Lake	0	5	2	0	1	0	22	0	8	0	14	0	0	52
Total	1	5	432	3	18	1	333	10	8	6	202	6	1	1026

		Locus – Oneμ2													
	206	208	212	222	226	240	242	244	246	248	250	252	254	256	258
Sashin Cr.sthd.	0	0	0	0	1	0	0	12	25	7	1	4	1	6	7
Sashin Cr. res.	0	0	0	0	0	0	0	12	36	8	20	10	3	22	5
Sashin Lake	0	0	0	0	0	0	0	0	34	11	9	25	2	9	7
Round Lake	0	0	0	0	0	4	0	0	2	5	7	7	8	28	0
Betty Lake	0	0	0	0	0	0	0	0	0	0	33	52	17	16	8
Fawn Lake	0	0	0	0	0	0	0	18	19	6	7	13	28	28	3
Rezanof Lake	0	0	0	0	0	0	3	1	24	1	15	13	4	24	0
Davidof Lake	0	0	0	0	0	0	0	0	11	1	29	6	6	13	1
Deer Lake	3	3	12	7	0	0	1	24	0	0	0	2	0	0	0
Total	3	3	12	7	1	4	4	67	151	39	121	132	69	146	31

Sashin Cr.sthd.	2	2	7	3	1	3	0	10	2	8	4	2	1	1	110
Sashin Cr. res.	0	0	0	0	0	0	2	28	0	0	0	0	0	0	146
Sashin Lake	0	0	0	0	0	0	0	21	0	0	0	0	0	0	118
Round Lake	0	0	0	0	0	0	10	1	0	0	0	0	0	0	72
Betty Lake	0	0	0	0	0	0	0	0	0	0	0	0	0	0	126
Fawn Lake	0	0	0	0	0	0	0	4	0	0	0	0	0	0	126
Rezanof Lake	0	0	0	0	0	0	3	24	0	0	0	0	0	0	112
Davidof Lake	0	0	0	0	0	0	10	17	0	0	0	0	0	0	94
Deer Lake	0	0	0	0	0	0	0	0	0	0	0	0	0	0	52
Total	2	2	7	3	1	3	25	105	2	8	4	2	1	1	956

		Locus – Ssa14													
	130	132	134	136	138	140	142	144	146	148	150	152	154	Total	
Sashin Cr.sthd.	78	4	7	17	1	2	2	0	0	0	0	0	1	112	
Sashin Cr. res.	87	0	0	55	0	10	0	0	0	0	0	0	0	152	
Sashin Lake	64	0	1	43	0	6	0	0	0	0	0	0	0	114	
Round Lake	43	0	39	33	0	0	0	0	1	0	0	0	0	116	
Betty Lake	83	0	0	39	0	0	0	0	0	0	0	0	0	122	
Fawn Lake	105	0	0	17	0	8	0	0	0	0	0	0	0	130	
Rezanof Lake	71	0	0	59	0	0	0	0	0	0	0	0	0	130	
Davidof Lake	32	0	0	61	0	1	0	0	0	0	0	0	0	94	
Deer Lake	27	0	1	0	0	0	0	9	0	1	11	1	0	50	
Total	590	4	48	324	1	27	2	9	1	1	11	1	1	1020	

		Locus – Omy325															
	95	97	99	101	103	105	109	111	113	123	125	127	129	131	133	135	Total
Sashin Cr.sthd.	2	4	2	0	1	30	48	0	3	1	0	5	0	0	13	3	112
Sashin Cr. res.	0	0	0	0	0	52	52	0	0	0	0	1	0	0	45	0	150
Sashin Lake	0	0	0	0	0	44	44	0	0	0	0	3	0	0	25	0	116
Round Lake	0	0	0	0	0	30	51	0	0	0	0	8	2	0	27	0	118
Betty Lake	0	0	0	0	1	65	0	0	0	0	0	0	0	0	58	0	124
Fawn Lake	0	0	0	0	0	22	65	0	0	0	1	13	12	1	8	0	122
Rezanof Lake	0	6	0	0	0	96	23	0	0	0	0	6	0	2	1	0	134
Davidof Lake	0	0	0	0	0	39	37	0	0	0	0	0	0	2	18	0	96
Deer Lake	0	0	0	1	0	3	9	1	13	10	10	0	1	4	0	0	52
Total	2	10	2	1	2	381	329	1	16	11	11	36	15	9	195	3	1024

Appendix 2. Allozyme variation in Oncorhychus mykiss from southern Baranof Island, Southeast Alaska. Alleles are designated by their mobility relative to the most common allele (**100*) and described in Shaklee et. al. (1990).

Location	N	*mAAT-2**		N	*sAAT-4**			N	*ADA-1**		N	*ADA-2**		N	*ADH**		N	*sAH**		N	*BGLUA**		
		**-100*	**-90*		**100*	**69*	**80*		**100*	**85*		**100*	**90*		**100*	**-128*		**100*	**85*		**100*	**90*	**50*
Sashin steelhead	43	1.000	0.000	39	0.987	0.013	0.000	43	1.000	0.000	43	1.000	0.000	43	1.000	0.000	43	0.814	0.186	43	0.965	0.000	0.035
Sashin Lake	60	1.000	0.000	54	1.000	0.000	0.000	60	1.000	0.000	60	0.992	0.008	60	1.000	0.000	58	0.672	0.328	60	1.000	0.000	0.000
Sashin Cr. Res.	71	1.000	0.000	70	0.986	0.014	0.000	71	1.000	0.000	71	1.000	0.000	71	1.000	0.000	70	0.743	0.257	71	1.000	0.000	0.000
Round Lake	81	1.000	0.000	69	0.826	0.174	0.000	80	1.000	0.000	80	1.000	0.000	81	1.000	0.000	81	0.586	0.414	81	1.000	0.000	0.000
Betty Lake	60	1.000	0.000	59	1.000	0.000	0.000	60	1.000	0.000	60	1.000	0.000	60	1.000	0.000	60	0.992	0.008	60	1.000	0.000	0.000
Davidof Lake	60	1.000	0.000	58	1.000	0.000	0.000	60	1.000	0.000	60	1.000	0.000	60	0.992	0.008	59	0.797	0.203	60	1.000	0.000	0.000
Fawn Lake	60	1.000	0.000	51	0.951	0.049	0.000	60	1.000	0.000	60	1.000	0.000	60	1.000	0.000	57	0.868	0.132	60	1.000	0.000	0.000
Rezanof Lake	60	1.000	0.000	56	0.777	0.223	0.000	60	1.000	0.000	60	1.000	0.000	60	1.000	0.000	60	0.983	0.017	60	1.000	0.000	0.000
Deer Lake	57	0.851	0.149	56	0.607	0.116	0.277	57	0.640	0.360	57	1.000	0.000	57	1.000	0.000	52	0.990	0.010	57	0.816	0.096	0.088

	N	*CK-C2**		N	*FH**		N	*G3PDH-3**		N	*G3PDH-4**			N	*mIDHP-2**		N	*sIDHP-1,2**				N	*LDH-B2**	
		**100*	**104*		**100*	**84*		**100*	**121*		**100*	**124*	**85*		**100*	**144*		**100*	**42*	**121*	**72*		**100*	**76*
Sashin steelhead	43	0.988	0.012	43	1.000	0.000	43	0.977	0.023	43	0.430	0.558	0.012	43	0.860	0.140	43	0.465	0.215	0.110	0.209	43	0.942	0.058
Sashin Lake	60	0.933	0.067	60	1.000	0.000	60	1.000	0.000	60	0.192	0.808	0.000	60	1.000	0.000	60	0.446	0.192	0.171	0.192	60	1.000	0.000
Sashin Cr. Res.	71	1.000	0.000	71	1.000	0.000	71	1.000	0.000	71	0.148	0.852	0.000	70	1.000	0.000	71	0.359	0.204	0.162	0.275	71	1.000	0.000
Round Lake	80	0.994	0.006	81	1.000	0.000	79	1.000	0.000	78	0.006	0.994	0.000	81	1.000	0.000	81	0.494	0.204	0.191	0.111	81	1.000	0.000
Betty Lake	60	1.000	0.000	60	1.000	0.000	58	1.000	0.000	58	0.440	0.560	0.000	60	1.000	0.000	60	0.492	0.238	0.088	0.183	60	1.000	0.000
Davidof Lake	60	1.000	0.000	60	1.000	0.000	60	1.000	0.000	60	0.350	0.650	0.000	60	1.000	0.000	60	0.542	0.229	0.104	0.125	60	1.000	0.000
Fawn Lake	60	1.000	0.000	60	1.000	0.000	60	1.000	0.000	59	0.059	0.941	0.000	60	1.000	0.000	59	0.530	0.246	0.072	0.153	60	1.000	0.000
Rezanof Lake	60	1.000	0.000	60	0.992	0.008	60	1.000	0.000	60	0.000	1.000	0.000	60	1.000	0.000	60	0.425	0.204	0.233	0.138	60	1.000	0.000
Deer Lake	57	0.991	0.009	57	1.000	0.000	57	1.000	0.000	57	0.044	0.912	0.044	57	0.974	0.026	57	0.667	0.202	0.000	0.132	57	1.000	0.000

	N	*sMDH-A1,2**		N	*sMDH-B1,2**		N	*sMEP-1**		N	*PEPD-1**		N	*PGK-2**				N	*PGM-1**		N	*sSOD**	
		**100*	**155*		**100*	**120*		**100*	**83*		**100*	**110*		**100*	**115*	**144*	**90*		**100*	**Null*		**100*	**226*
Sashin steelhead	43	1.000	0.000	43	0.942	0.058	43	0.779	0.221	42	0.988	0.012	43	0.523	0.465	0.000	0.012	43	1.000	0.000	43	0.767	0.233
Sashin Lake	60	1.000	0.000	60	1.000	0.000	60	0.533	0.467	60	1.000	0.000	60	0.000	1.000	0.000	0.000	60	1.000	0.000	60	0.817	0.183
Sashin Cr. Res.	71	1.000	0.000	71	1.000	0.000	71	0.599	0.401	68	1.000	0.000	71	0.000	0.993	0.000	0.007	71	1.000	0.000	71	0.789	0.211
Round Lake	81	1.000	0.000	81	1.000	0.000	79	0.494	0.506	67	1.000	0.000	81	0.000	1.000	0.000	0.000	81	1.000	0.000	81	0.926	0.074
Betty Lake	60	1.000	0.000	60	1.000	0.000	60	0.600	0.400	59	1.000	0.000	60	0.000	1.000	0.000	0.000	60	1.000	0.000	60	0.575	0.425
Davidof Lake	60	1.000	0.000	60	1.000	0.000	60	0.417	0.583	60	1.000	0.000	60	0.000	1.000	0.000	0.000	60	0.967	0.033	60	0.433	0.567
Fawn Lake	60	1.000	0.000	60	1.000	0.000	60	0.633	0.367	59	1.000	0.000	60	0.000	1.000	0.000	0.000	60	1.000	0.000	60	0.600	0.400
Rezanof Lake	60	1.000	0.000	60	1.000	0.000	60	0.692	0.308	55	1.000	0.000	60	0.000	1.000	0.000	0.000	60	1.000	0.000	60	0.692	0.308
Deer Lake	57	1.000	0.000	57	0.956	0.044	57	1.000	0.000	57	1.000	0.000	57	0.184	0.798	0.018	0.000	57	1.000	0.000	57	0.833	0.167

N refers to number of fish sampled.

Appendix 3. Sample sizes of monomorphic loci screened.

Location	*mAAT-1*	*sAAT-1,2*	*sAAT-3*	*mAH-1*	*mAH-2*	*mAH-3*	*mAH-4*	*AK*	*ALAT*	*BGALA*	*CKA1*	*CKA2*	*CKB*	*CKC!*
Sashin steelhead	43	43	43	40	40	40	32	43	40	43	43	43	43	43
Sashin Lake	60	60	60	60	60	60	60	60	57	60	60	60	60	60
Sashin Cr. Res.	71	71	71	71	71	71	71	71	70	71	71	71	71	71
Round Lake	60	60	60	60	60	60	60	60	59	60	60	60	60	60
Betty Lake	60	60	60	59	59	59	59	60	60	60	60	60	60	60
Davidof Lake	60	60	60	60	60	60	60	60	53	60	60	60	60	60
Fawn Lake	60	60	60	60	60	60	60	60	60	60	60	60	60	60
Rezanof Lake	81	81	81	81	81	81	81	30	67	81	81	81	81	81
Deer Lake	57	57	57	57	57	57	57	57	57	57	57	57	57	57

Location	*ESTD*	*FDHG*	*GAPDH-2*	*GAPDH-3*	*GAPDH-4*	*GR*	*GPIA*	*GPI-B1*	*GPI-B2*	*G3PDH-1*	*G3PDH-2*	*IDDH-1*	*IDDH-2*	*mIDHP-1*
Sashin steelhead	43	43	43	41	43	43	43	43	43	43	43	43	43	43
Sashin Lake	59	60	60	60	60	60	59	59	59	60	60	60	60	60
Sashin Cr. Res.	71	71	71	71	71	69	71	71	70	71	71	71	71	70
Round Lake	69	81	81	79	81	81	81	81	81	81	81	81	81	81
Betty Lake	60	60	59	59	59	59	60	60	60	60	60	60	60	60
Davidof Lake	58	60	60	60	60	60	60	60	60	60	59	60	60	60
Fawn Lake	60	60	60	60	60	60	60	60	60	60	60	60	60	60
Rezanof Lake	59	60	60	60	60	60	60	60	60	60	60	60	60	60
Deer Lake	57	57	57	57	57	57	57	57	57	57	57	57	57	57

Location	*LDH-B1*	*LDH-C*	*mMDH-1*	*mMDH-2*	*mMDH-3*	*sMDH-A1*	*sMDH-B1*	*mMEP-1*	*MPI*	*PEPA*	*PEPB-1*	*PEPLT*	*PGDH*	*PGM-R*
Sashin steelhead	43	43	43	41	43	43	43	43	43	43	42	43	43	43
Sashin Lake	60	60	60	60	60	60	60	60	60	58	60	60	60	60
Sashin Cr. Res.	71	71	71	71	71	71	71	71	71	71	70	67	69	71
Round Lake	81	81	81	81	81	81	81	81	81	74	80	81	81	81
Betty Lake	60	60	60	60	60	60	60	60	60	60	60	60	60	60
Davidof Lake	60	60	60	60	60	60	60	60	60	60	60	60	60	60
Fawn Lake	60	60	60	60	60	60	60	60	60	60	60	60	60	60
Rezanof Lake	60	60	60	60	60	60	60	60	60	60	60	60	60	60
Deer Lake	57	57	57	57	57	57	57	57	57	57	57	57	57	57

Location	*PGM-2*	*PNP*	*mSOD*	*TPI-1*	*TPI-2*	*TPI-3*	*TPI-4*
Sashin steelhead	43	19	43	43	43	43	43
Sashin Lake	60	60	59	60	60	60	60
Sashin Cr. Res.	71	71	71	71	71	71	71
Round Lake	81	75	50	81	81	81	81
Betty Lake	60	60	59	60	60	60	60
Davidof Lake	60	60	60	60	60	60	60
Fawn Lake	60	60	60	60	59	60	60
Rezanof Lake	60	60	60	60	60	60	60
Deer Lake	57	57	57	57	57	57	57